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Short communication

Detection of a spotted fever group *Rickettsia* in the tick *Haemaphysalis juxtakochi* in Rondonia, Brazil

Marcelo B. Labruna[a,b,*], Luis Marcelo A. Camargo[c], Erney P. Camargo[c], David H. Walker[a]

[a]*Departamento de Medicina Veterinaria Preventiva e Saude Animal, Faculdade de Medicina Veterinaria e Zootenia, Universidade de Sao Paulo, USP/FMVZ-Av. Prof. Orlando Marques de Paiva, 87, Sao Paulo, SP 05508-000, Brazil*
[b]*Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555-0609, USA*
[c]*Departamento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo, SP 05508-000, Brazil*

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Abstract

The tick genus *Haemaphysalis* is represented by four species in the New World, of which only the species *Haemaphysalis leporispalustris* has been associated with Rickettsiae. The present study reports for the first time the presence of a *Rickettsia* strain in the tick *Haemaphysalis juxtakochi.* A free-living male of *H. juxtakochi*, collected in the state of Rondonia, Western Amazon, Brazil, was subjected to DNA extraction and tested by PCR targeting the four rickettsial genes: *gltA*, 17-kDa, *ompA* and *ompB*. The nucleotide sequences obtained from the PCR products were, by BLAST analyses, closest to *Rickettsia rhipicephali* sharing 99.7% (1147/1150), 98.8% (429/434), 99.0% (486/491) and 99.0% (809/817) identities with the partial sequences of the *gltA*, 17-kDa, *ompA* and *ompB* genes, respectively. Phylogenetic analyses inferred from the four rickettsial genes showed a high-degree of similarity of this *H. juxtakochi–Rickettsia* with *R. rhipicephali.* These two agents grouped together in all trees, always with high bootstrap support (75–96%). This study gives molecular evidence for the presence of a *Rickettsia* species, designated as strain R300, in the tick *H. juxtakochi* from the Western Amazon area of Brazil. Genetic analyses showed R300 to be closely related to *R. rhipicephali*.

Keywords: *Rickettsia*; *Haemaphysalis juxtakochi*; Ticks; Amazon; Brazil

1. Introduction

Rickettsiae are obligate intracellular bacteria, which have been divided in three groups: (1) the ancestral group, composed of *Rickettsia bellii* and *Rickettsia canadensis,* is associated with ticks, (2) the

* Corresponding author. Tel.: +55 11 30917701; fax: +55 11 30917928.
E-mail address: labruna@usp.br (M.B. Labruna).

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typhus group, composed of *Rickettsia prowazekii* and *Rickettsia typhi*, is associated with lice and fleas, (3) and the spotted fever group (SFG), which includes more than 20 valid species, is mostly associated with ticks but at least one species is associated with mites and another one with fleas (Stothard et al., 1994; Roux et al., 1997; Yu and Walker, 2003). In all continents except Antarctica, many rickettsiae have been described as agents of important human diseases. Some *Rickettsia* species (e.g., *Rickettsia conorii, Rickettsia sibirica*) are distributed in more than one continent whereas others (*R. prowazekii, R. typhi, Rickettsia felis*) have a worldwide distribution (Weiss and Moulder, 1984; La Scola et al., 2002).

Most of the known tick-associated *Rickettsia* species found in the North, Central and South Americas are endemic to these continents. The only known exception is *Rickettsia africae*, which is originally from Africa but has been accidentally introduced within its vector *Amblyomma variegatum* into Guadaloupe (West Indies) (Parola et al., 1999). At least nine SFG species have been associated with ticks found exclusively in the American continents, with the vast majority reported from North America (Weiss and Moulder, 1984; Labruna et al., 2004c), probably because much more research has been conducted in this region than in Latin America. At the beginning of this century, the only tick-borne *Rickettsia* known to occur in South America was *Rickettsia rickettsii.* Recently, *R. bellii* and *Rickettsia amblyommii,* so far only reported in ticks from the United States, have been detected in different tick species of the genus *Amblyomma* in Brazil (Labruna et al., 2004a,c) and two additional SFG rickettsial strains, yet to be isolated and characterized, have also been found in *Amblyomma* ticks from Brazil (Labruna et al., 2004a,b).

In the Neotropical region, most ticks, including all the main anthropophilic ixodids, are in the genus *Ambylomma*. Therefore, most of the South American rickettsial studies have emphasized this genus and all the *Rickettsia* species reported from this region has been detected in *Amblyomma* species. The genus *Haemaphysalis* is represented by only four species in the American continents (Keirans, 1992) and only the species *Haemaphysalis leporispalustris* has been associated with rickettsiae, namely *R. rickettsii* and *R. canadensis* in North America (Parker et al., 1951; McKiel et al., 1967). The present study reports for the first time the presence of a *Rickettsia* strain in the tick *Haemaphysalis juxtakochi.*

2. Materials and methods

In December 2001, during a survey on ticks of the genus *Amblyomma* in Brazil (Labruna et al., 2004c), a single adult *H. juxtakochi* male tick was collected on the vegetation in the headwaters of the Jaguari River (10°17′S, 63°14′W), Monte Negro Municipality, state of Rondonia, Western Amazon, Brazil. The tick was brought alive to the laboratory and was frozen at −80 °C until processed. The tick was thawed at room temperature and processed for DNA extraction as described (Labruna et al., 2004a). Polymerase chain reaction (PCR) assays targeting the rickettsial genes for citrate synthase (*gltA*) (Labruna et al., 2004a), outer membrane protein A (*ompA*), outer membrane protein B (*ompB*), and the 17-kDa genus-specific protein gene were employed following the protocols described previously (Labruna et al., 2004b). PCR products of the expected size were cloned and sequenced in an automated DNA sequencer (Labruna et al., 2004a).

Partial DNA sequences obtained by sequencing the PCR products were submitted to BLAST analysis (Altschul et al., 1990) to determine similarities to other *Rickettsia* species. The sequences obtained were aligned for each gene (*gltA*, 17-kDa, *ompA,* and *ompB*) with the corresponding sequences of other *Rickettsia* species available in GenBank using the CLUSTAL algorithm of the program MegAlign (DNAstar, Lasergene, Madison, WI). Available sequences of other Brazilian rickettsial isolates (*R. bellii* and strain COOPERI (Labruna et al., 2004a), *R. amblyommii* (Labruna et al., 2004c), and strain Aranha (Labruna et al., 2004b) were included in the phylogenetic analysis. Phylogenetic relationships were inferred by using PAUP 4.0b1 (Swofford, 1999). For each gene analyzed, a phylodendogram was constructed by the neighbor-joining method, using Kimura's two-parameter model. Confidence values for individual branches of the resulting tree were determined by bootstrap analysis with 1000 replicates.

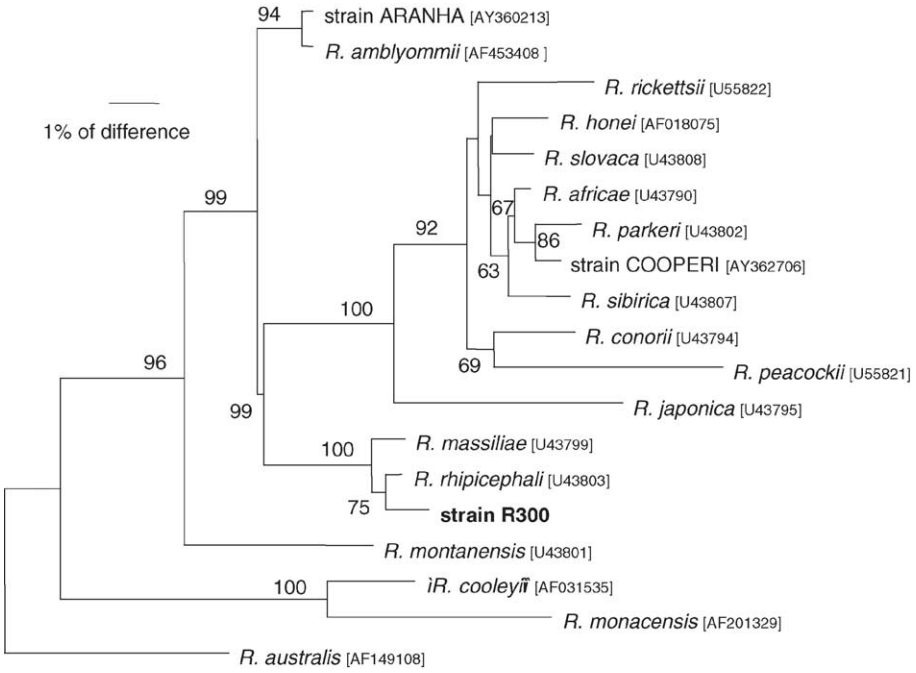

Fig. 1. Neighbor-joining phylodendogram based on partial *ompA* sequences showing the phylogenetic placement of strain R300 among SFG rickettsial species. Bootstrap support (>50%) for phylogenetic groupings and the scale of percent difference between taxa are indicated.

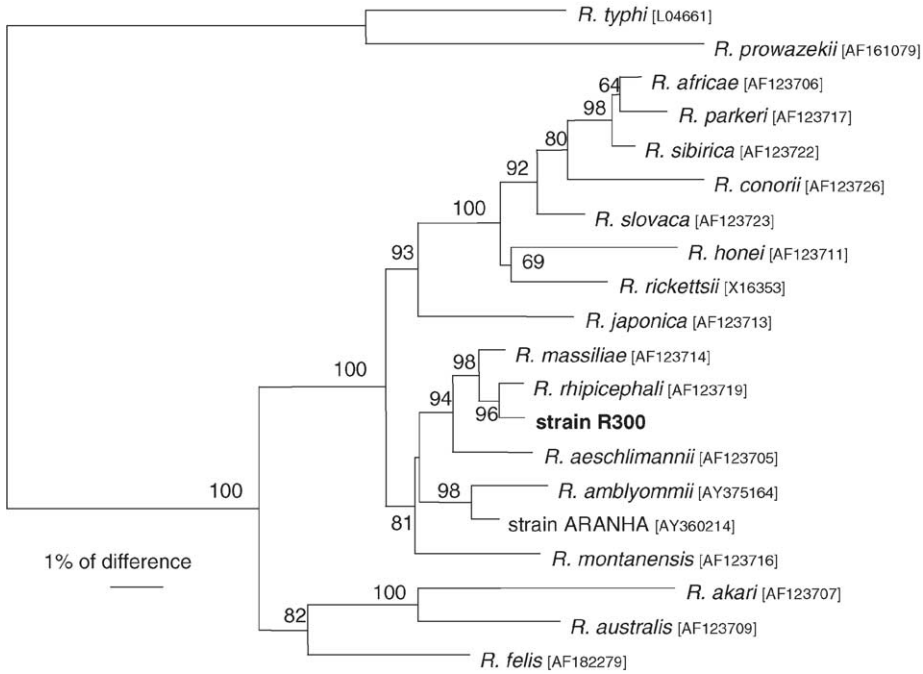

Fig. 2. Neighbor-joining phylodendogram based on partial *ompB* sequences showing the phylogenetic placement of strain R300 among rickettsial species. Bootstrap support (>50%) for phylogenetic groupings and the scale of percent difference between taxa are indicated.

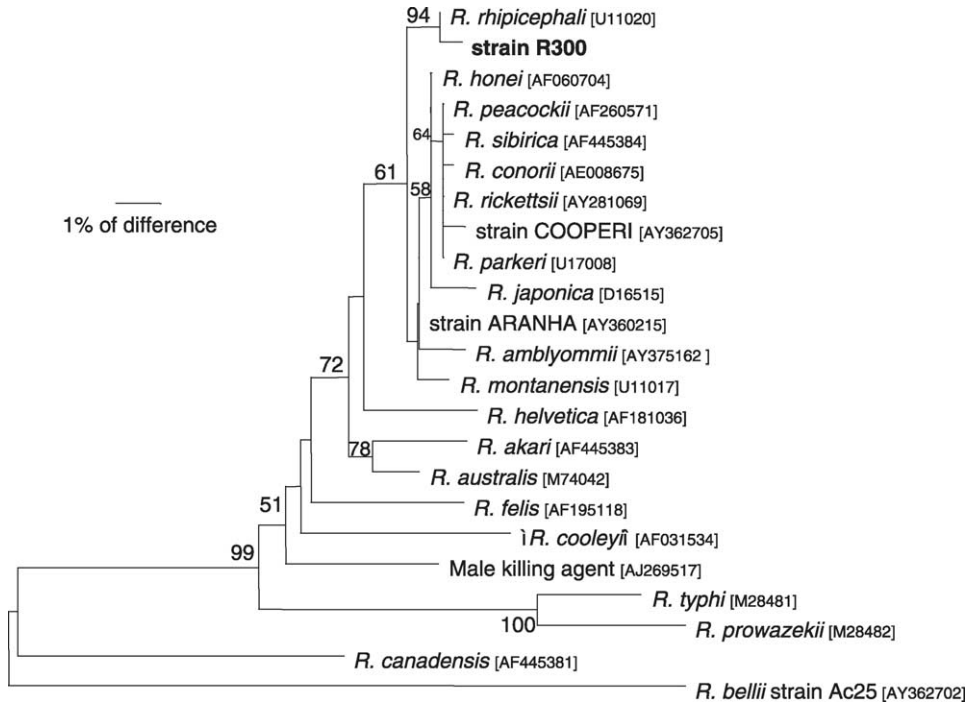

Fig. 3. Neighbor-joining phylodendogram based on partial 17-kDa sequences showing the phylogenetic placement of strain R300 among validated rickettsial species. Bootstrap support (>50%) for phylogenetic groupings and the scale of percent difference between taxa are indicated.

3. Results

Amplification of four separate rickettsial gene sequences demonstrated the presence of rickettsiae in the *H. juxtakochi* tick. The *H. juxtakochi* tick was demonstrated to contain rickettsiae by PCR for the four rickettsial genes investigated. The DNA sequences obtained (excluding the primer sequences) for the genes *gltA*, 17-kDa, *ompA,* and *ompB* were 1150, 498, 491 and 818 bp, respectively, as expected for rickettsiae. Due to the limited partial sequence fragments of many rickettsiae available in GenBank, phylogenetic analyses were performed using 976, 393, 415, and 816 bp segments of the *gltA*, 17-kDa, *ompA,* and *ompB* genes, respectively. The *H. juxtakochi* rickettsial strain, which we designated as strain R300, belongs to the SFG since it contained the *ompA* gene (so far found only in SFG species) and clustered with other SFG species in all phylogenetic trees (Figs. 1–4). By BLAST analysis, the closest *Rickettsia* species to R300 was *Rickettsia rhipicephali,* sharing 99.7% (1147/1150), 98.8% (429/434), 99.0% (486/491) and 99.0% (809/817) of similarities between the partial sequences of the genes *gltA*, 17-kDa, *ompA,* and *ompB*, respectively.

Phylogenetic analyses inferred from the four rickettsial genes showed a high degree of similarity of strain R300 with *R. rhipicephali.* These two agents grouped together in all trees, always with high bootstrap support (75–96%) (Figs. 1–4). In all phylogenetic analyses, the Kimura distance values between the partial sequences of strain R300 and *R. rhipicephali* were similar to the lowest values found between the closest *Rickettsia* species.

The GenBank nucleotide sequence accession numbers for the gene partial sequences of strain R300, generated in this study are AY472040 for *ompA* gene, AY472041 for the *ompB* gene, AY472039 for 17-kDa gene, and AY472038 for *gltA* gene.

4. Discussion

This study provides molecular evidence for the infection of a *Rickettsia* species, designated as strain

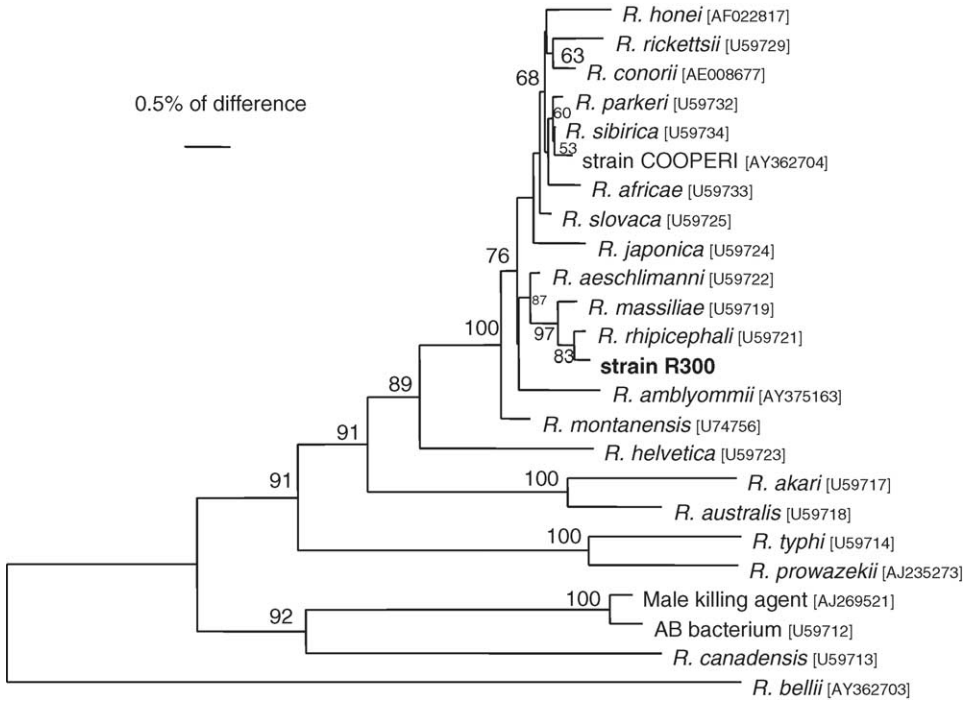

Fig. 4. Neighbor-joining phylodendogram based on partial *gltA* sequences showing the phylogenetic placement of strain R300 among validated rickettsial species. Bootstrap support (>50%) for phylogenetic groupings and the scale of percent difference between taxa are indicated.

R300, in the tick *H. juxtakochi* from the Western Amazon area of Brazil. Genetic analyses of four rickettsial genes showed R300 to be closely related to *R. rhipicephali.* As the genetic distances between R300 and *R. rhipicephali* inferred by the four rickettsial genes were among the lowest ones found among the SFG rickettsiae, it is quite suggestive that R300 is a strain of *R. rhipicephali* in South America. This statement is reinforced by recent studies that have suggested that the most closely related SFG *Rickettsia* species should in fact, be considered different geographic strains of a single species (Stenos and Walker, 2000; Roux and Raoult, 2000). Finally, we exclude the possibility of DNA contamination in our sample because we have not worked with *R. rhipicephali* in the laboratory.

R. rhipicephali was first discovered in the tick *Rhipicephalus sanguineus* from Mississippi, United States (Burgdorfer et al., 1975). Subsequently, it was isolated in the same country from the ticks *Dermacentor andersoni, Dermacentor variabilis,* and *Dermacentor occidentalis* (Burgdorfer et al., 1978; Philip and Casper, 1981; Philip et al., 1981). There is no evidence that *R. rhipicephali* is pathogenic for humans. In the laboratory, *R. rhipicephali* was moderately pathogenic for guinea pigs (Burgdorfer et al., 1978). The possible presence of *R. rhipicephali* in South America, as suggested by our results, is not a total surprise, since all other *Rickettsia* species detected in ticks in Brazil (*R. rickettsii, R. bellii, R. amblyommii*) have also been found in ticks in USA (Philip et al., 1978; Labruna et al., 2004a,c). Despite the fact that most of the tick species from North (Nearctic) and South (Neotropic) Americas are quite distinct, the *Rickettsia* species among them have been shown to be very similar.

This is the first report of a *Rickettsia* species in the tick *H. juxtakochi.* This tick is distributed from Mexico to Argentina. Although deer are likely to be its main primary host, *H. juxtakochi* has been reported on several mammalian species, including tapirs, rodents, lagomorphs and non-human primates and also on birds (Jones et al., 1972; Beldomenico et al., 2003). Further studies should attempt the isolation of this rickettsial

strain from *H. juxtakochi* ticks to evaluate its phenotypic and other genotypic characteristics, in order to confirm its taxonomic status.

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